

5 (a) incubating said sample with a mixture of particles in a first suspension,

6 said mixture of particles comprised of groups (i) through (iv):

7 (i) particles coated with anti-thyroid stimulating hormone,

8 (ii) particles coated with anti-triiodothyronine,

9 (iii) particles coated with anti-thyroxine, and

10 (iv) particles coated with a mixture of a diluting agent and a member
11 selected from the group consisting of thyroid peroxidase and
12 anti-human IgG,

13 the particles of each group distinguishable from the particles of each
14 other group by a flow cytometry distinguishable characteristic that is
15 independent of the coatings of subparagraphs (i), (ii), (iii), and (iv);

16 (b) recovering said particles from said first suspension, and incubating
17 said recovered particles with a mixture of labeled binding members in
18 a second suspension, said mixture of labeled binding members
19 comprising:

20 (1) labeled anti-thyroid stimulating hormone,

21 (2) a labeled analog composition toward which anti-triiodothyronine
22 and anti-thyroxine have immunological binding affinity, but in
23 which said immunological binding affinity is less than that of
24 anti-triiodothyronine toward triiodothyronine and of anti-
25 thyroxine toward thyroxine, and

26 (3) either labeled anti-human IgG when particles of group (iv) are
27 coated with thyroid peroxidase, or labeled thyroid peroxidase
28 when particles of group (iv) are coated with anti-human IgG;

29 said diluting agent being inert toward said biological markers and said labeled
30 binding members; and

31 (c) recovering said particles from said second suspension and detecting
32 the amount of label bound to said particles thus recovered from said
33 second suspension while correlating by flow cytometry the amount of

A1
cm's

B1